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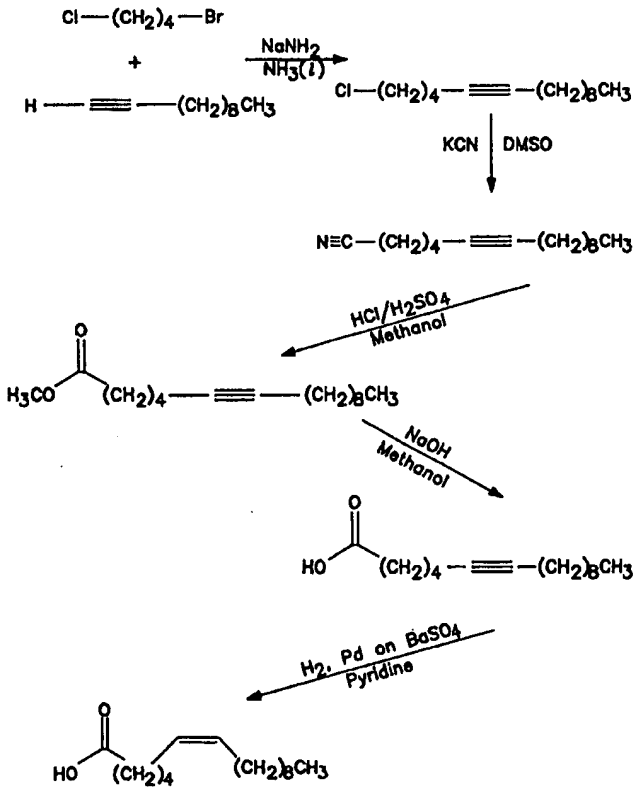
(54) Title: ANTIMICROBIAL LIPIDS

(57) Abstract

The invention relates to compositions of skin-derived lipids that inhibit the ability of microorganisms to adhere to skin, inhibit the reproduction of microorganism and are toxic to microorganisms. The invention icludes the two fatty acids shown below and the derivatives or analogs thereof as shown in the figure.



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ANTIMICROBIAL LIPIDS

The present invention relates to compositions of certain antimicrobial skin lipids and to methods of preparing and using these lipids. The invention also relates to non-irritating compositions comprising these anti-microbial skin lipids.

The skin functions as a barrier between the outside world and the internal organs of the body. It serves the protective function of keeping harmful microbial organisms from colonizing the interior of an animal. Part of this protective function can be ascribed to the lipids present in epidermis, sebaceous secretions, or otherwise present at the skin surface. The distribution of lipid types at the skin differs markedly from the distribution found in internal organs. See, Nicolaides, *Science*, 186:19-26 (1974). Some of these skin-derived lipids have been reported to have pharmaceutical activity in inhibiting the growth of microbial organisms. See, Kabera et al., *Antimicrobial Agents and Chemotherapy*, 2:23-28 (1972). However, many of the fatty acids among these antimicrobial lipids are known to be extremely irritating.

Non-irritating natural lipid compositions that prevent infection or biological decay are desirable, since they are unlikely to cause adverse reactions. Therefore, what is needed in the art are such compositions formulated with non-irritating lipids or such compositions wherein the antimicrobial action of the lipids is accentuated so that the lipids can be present at concentrations less than the threshold concentration for irritation.

Summary of the Invention

The invention is described below with reference to the following formula:

(5) $CH_3(CH_2)_a-\overset{H}{\underset{|}{C}}=\overset{H}{\underset{|}{C}}-(CH_2)_b-R^5$;

wherein a + b equals from 11 to 14 and b is an integer from 1 to 14, wherein R^5 is (a) $CH_2NR^{21}R^{22}$, wherein R^{21} and R^{22} are independently hydrogen or C1 to C6 hydrocarbon, preferably C1 to C4 hydrocarbon, (b) C(O)-R^{23}, R^{23} is (i) $NR^{24}R^{25}$, wherein R^{24} and R^{25} are independently hydrogen or C1 to C6 hydrocarbon, preferably C1 to C4 hydrocarbon, (ii) OR^{26}, wherein R^{26} is glyceryl or a glyceryl fatty acid ester, wherein said fatty acid is about C15 to about C18, or (iii) a hydroxyl, or (c) CH_2OH. The invention is further described with reference to the following formulas of preferred compounds:

(1) $CH_3(CH_2)_8-\overset{H}{\underset{|}{C}}=\overset{H}{\underset{|}{C}}-(CH_2)_4-R^1$;

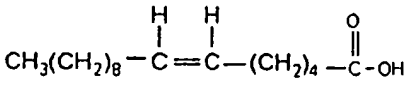
(2) $CH_3(CH_2)_8-\overset{H}{\underset{|}{C}}=\overset{H}{\underset{|}{C}}-(CH_2)_6-R^2$;

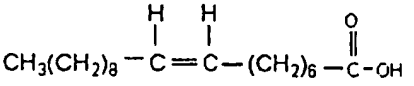
(3) $CH_3(CH_2)_5-\overset{H}{\underset{|}{C}}=\overset{H}{\underset{|}{C}}-(CH_2)_7-R^3$;

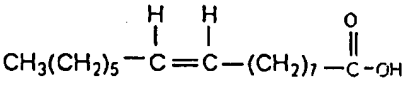
(4) $CH_3(CH_2)_8-\overset{H}{\underset{|}{C}}=\overset{H}{\underset{|}{C}}-(CH_2)_n-R^4$;

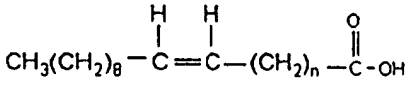
wherein n is an integer from 3 to 6 and R^1, R^2, R^3 and R^4 are independently (a) $CH_2NR^{11}R^{12}$, wherein R^{11} and R^{12} are independently hydrogen or C1 to C6 hydrocarbon, preferably C1 to C4 hydrocarbon, (b) C(O)-R^{13},wherein R^{13} is (i) $NR^{14}R^{15}$, wherein R^{14} and R^{15} are independently hydrogen or C1 to C6 hydrocarbon, preferably C1 to C4 hydrocarbon, (ii) OR^{16}, wherein R^{16} is glyceryl or a glyceryl fatty acid ester, wherein said fatty acid is about C15 to about C18, or (iii) a hydroxyl, or (c) CH_2OH. The hydrocarbon moieties referred to herein are preferably alkyl.

The formulas below describing fatty acid compounds are helpful to understanding the invention:

(1a)  [C16:1Δ6]

(2a)  [C18:1Δ8]

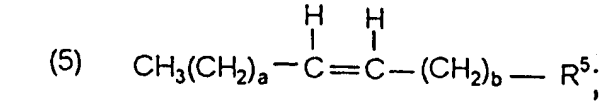
(3a)  [C16:1Δ9] [Palmitoleic]

(4a) 

The above formulas refer to the cis configuration of the carbon-carbon double bond. C16:1Δ6 and C18:1Δ8 are believed to be derived from sebum, i.e., the secretions from sebaceous glands.

All of fatty acids (1a)-(4a) described above have antimicrobial activity, particularly against a broad spectrum of gram positive bacteria, but also against other microorganisms including a number of gram negative bacteria. They further inhibit the adherence of bacteria and fungi to animal skin. Additionally, they are believed to be active against lipid-coated viral particles. C16:1Δ6 and C16:1Δ9 were surprisingly found to be non-irritating, even at concentrations as high a 10% w/v. These results were for example obtained by contacting skin with the fatty acid in a hydroxypropyl methylcellulose gel and in the presence of alcohol as high as 75%. Surprisingly, compounds of the formulas (1) - (5) have been shown to been found to have much greater antimicrobial activity when combined with an alcohol or a polyalkylene glycol.

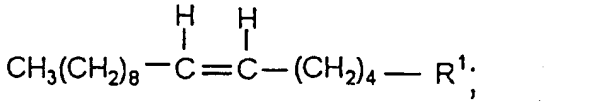
In a first embodiment, the invention provides an antimicrobial composition comprising (A) antimicrobial activity enhancing effective amount of a component selected from the group consisting of (C2-C7) alkyl alcohols, poly(alkylene oxide)s wherein the alkylene moieties are C2 to C4, and mixtures thereof; and (B) a compound as follows:

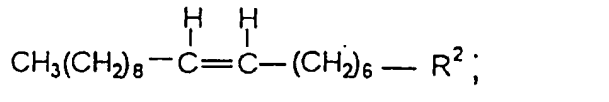
(5) 

wherein a + b equals from 11 to 14 and b is an integer from 1 to 14,

wherein R^5 is (a) $CH_2NR^{21}R^{22}$, wherein R^{21} and R^{22} are independently hydrogen or C1 to C6 hydrocarbon, (b) C(O)-R^{23}, R^{23} is (i) $NR^{24}R^{25}$, wherein R^{24} and R^{25} are independently hydrogen or C1 to C6 hydrocarbon, (ii) OR^{26}, wherein R^{26} is glyceryl or a glyceryl fatty acid ester, wherein said fatty acid is about C15 to about C18, or (iii) a hydroxyl, or (c) CH_2OH, or a pharmaceutically acceptable salt thereof.

In a second embodiment, the invention provides an antimicrobial composition comprising (I) a pharmaceutically acceptable excipient and (II) an isolated compound of formula:

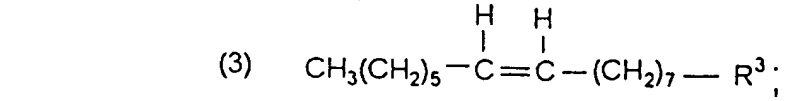
(1) 

(2) 

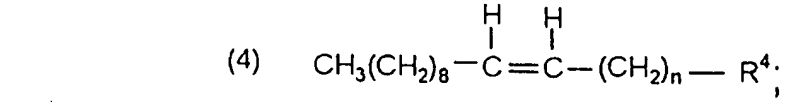
wherein R^1 and R^2 are independently (a) $CH_2NR^{11}R^{12}$, wherein R^{11} and R^{12} are independently hydrogen or C1 to C6 hydrocarbon, (b) C(O)-R^{13},wherein R^{13} is (i) $NR^{14}R^{15}$, wherein R^{14} and R^{15} are independently hydrogen or C1 to C6 hydrocarbon, (ii) OR^{16}, wherein R^{16} is glyceryl or a glyceryl fatty acid ester, wherein said fatty acid is about C15 to about C18, or (iii) a hydroxyl, or (c) CH_2OH, or a pharmaceutically acceptable salt thereof.

The compositions of the first and second embodiments have a number of preferred or alternative embodiments. In one

preferred embodiment, the compound is according to one of the following formulas

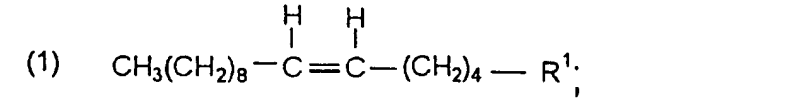


(3) $CH_3(CH_2)_5-C(H)=C(H)-(CH_2)_7-R^3$;

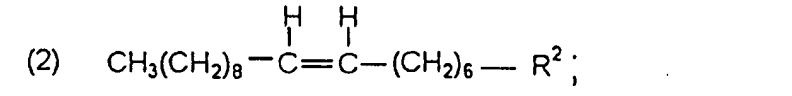


(4) $CH_3(CH_2)_8-C(H)=C(H)-(CH_2)_n-R^4$;

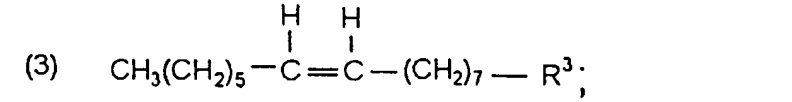
and mixtures thereof, wherein n is an integer from 3 to 6 and R^3 and R^4 are independently as set forth for R^5, or a pharmaceutically acceptable salt thereof. Preferably, the compound is of one of the following formulas



(1) $CH_3(CH_2)_8-C(H)=C(H)-(CH_2)_4-R^1$;



(2) $CH_3(CH_2)_8-C(H)=C(H)-(CH_2)_6-R^2$;



(3) $CH_3(CH_2)_5-C(H)=C(H)-(CH_2)_7-R^3$;

wherein R^1, R^2 and R^3 are independently (d) $CH_2NR^{21}R^{22}$, (e) $C(O)\text{-}R^{13*}$, wherein R^{13*} is (i) OR^{26} or (ii) a hydroxyl, or (f) CH_2OH, or a pharmaceutically acceptable salt thereof. In one embodiment, the compound is an ester with a monoglyceride having a mono- or di-unsaturated fatty acyl component. In another embodiment, the compound comprises an acid whereby R^1, R^2 or R^3 comprises COOH, or a pharmaceutically acceptable salt thereof. In another embodiment, the salt comprises a sodium, ammonium, silver, copper, calcium, barium, zinc or mono-, di- , tri- or quaternary alkylammonium salt, wherein said alkyl substituents on ammonium are independently C1 to C8. Preferably, the alkyl substituents on ammonium are independently C1 to C4 alkyl groups.

The compositions of the invention can be formulated for a device for dwelling on the skin, such as a wound dressing, ostomy device, IV tape, or the like. Preferably, such devices comprise a hydrocolloid gel.

The compositions of the invention can be adapted for application to an epithelial surface of a mammal. For instance, the composition can be a toothpaste, mouthwash, shampoo, hair styling composition, skin ointment, make-up composition, anti-oderant or antipersperant.

The compositions of the invention can be used to treat or prevent an infection of the gastrointestinal tract comprising administering an antimicrobially effective amount of an antimicrobial composition of the invention. In particular, the infection to be treated or prevented can be a *Helicobacter pylori* infection.

The compositions of the invention can be used to treat or prevent acne by administering an antimicrobially effective amount of an antimicrobial composition of the invention. Preferably, the amount of the antimicrobial composition administered is effective to kill or inhibit the replication of Propionibacterium.

The compositions of the invention can be used to preventing the adherence of a microbe to epithelial surfaces comprising administering a microbe adherence reducing effective amount of such an antimicrobial composition.

The invention further provides a lipid-replenishing skin treatment ointment comprising: (a) the antimicrobial composition of claim 1, and (b) a plurality of lipids selected from the lipids normally present on skin.

The compositions of the invention can be adapted for topical or oral administration, such as a cream adapted for topical administration.

In a third embodiment, the invention provides a method of treating or preventing infection, the method comprising applying or administering to an animal a composition comprising an antimicrobially effective amount of a compound selected from the group consisting of:

(4) $CH_3(CH_2)_8-\overset{H}{\underset{|}{C}}=\overset{H}{\underset{|}{C}}-(CH_2)_n-R^4$;

wherein n is an integer from 3 to 6 and R^4 is (a) $CH_2NR^{31}R^{32}$, wherein R^{31} and R^{32} are independently hydrogen or C1 to C6 hydrocarbon, (b) $C(O)-R^{33}$, wherein R^{33} is (i) $NR^{34}R^{35}$, wherein R^{34} and R^{35} are independently hydrogen or C1 to C6 hydrocarbon, (ii) OR^{36}, wherein R^{36} is glyceryl or a glyceryl fatty acid ester, wherein said fatty acid is about C15 to about C18, or (iii) a hydroxyl, or (c) CH_2OH, or a pharmaceutically acceptable salt of said compound.

In a fourth embodiment, the invention provides a method of treating or preventing infection, comprising:

(A) administering or applying is to an epithelial surface of an animal a compound as follows:

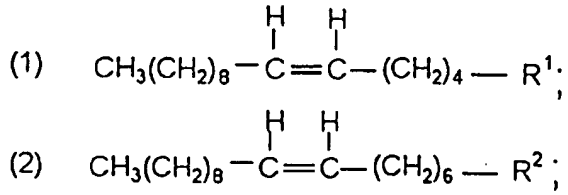
(5) $CH_3(CH_2)_a-\overset{H}{\underset{|}{C}}=\overset{H}{\underset{|}{C}}-(CH_2)_b-R^5$;

wherein a + b equals from 11 to 14 and b is an integer from 1 to 14,

wherein R^5 is as set forth above, or a pharmaceutically acceptable salt thereof; and

(B) applying or administering to the surface an antimicrobial activity enhancing effective amount of a component selected from the group consisting of (C2-C7) alkyl alcohols, poly(alkylene oxide)s wherein the alkylene moieties are C2 to C4, and mixtures thereof.

The methods of the third and fourth embodiments have a number of preferred or alternative embodiments. In one preferred embodiment, the compound applied or administered is according to one of the following formulas



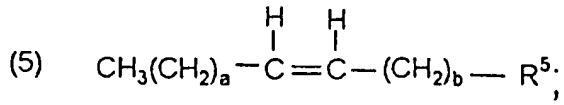
wherein R^1 and R^2 are independently (d) $CH_2NR^{21}R^{22}$, (e) $C(O)$-R^{13*}, or (f) CH_2OH, or a pharmaceutically acceptable salt thereof. All of the preferred or alternative embodiments described above for the compositions are, of course, applicable to these methods.

In one embodiment, the infection to be treated or prevented is caused by gram negative bacteria. In another embodiment, the infection to be treated or prevented is caused by a drug-resistant microbe, such as a drug-resistant bacteria, which can be a MRSA. In another embodiment, the infection to be treated or prevented is caused by a fungus.

The treatment and prevention methods of the invention can also be used to prevent the adherence of a microbe to epithelial surfaces.

In one preferred embodiment, the component is applied prior to the compound of formula (5).

In a fifth embodiment, the invention provides transdermal administration forms and methods. Thus, the invention provides a transdermal administration form for a biological agent comprising: (a) a bioactive agent; and (b) a transdermal transport effective amount of a compound as follows:



wherein a + b equals from 11 to 14 and b is an integer from 1 to 14, wherein R^5 is as set forth above, or a pharmaceutically acceptable salt thereof. The method of transdermal administration of a biological agent comprises the steps of: (a) topically applying a biological agent to a site on an animal; and (b) applying to the site a transdermal transport effective amount of a compound as follows:

(5) $CH_3(CH_2)_a-\overset{H}{\underset{|}{C}}=\overset{H}{\underset{|}{C}}-(CH_2)_b-R^5$;

wherein a + b equals from 11 to 14 and b is an integer from 1 to 14, wherein R^5 is as set forth above, or a pharmaceutically acceptable salt thereof. Preferably, the applying step (b) occurs prior to or concurrently with applying step (a).

In a sixth embodiment, the invention provides a preservation method and preserved materials. Accordingly, the invention provides a method of preserving a biologically degradable composition comprising contacting the composition with a preservation effective amount of a a compound as follows:

(5) $CH_3(CH_2)_a-\overset{H}{\underset{|}{C}}=\overset{H}{\underset{|}{C}}-(CH_2)_b-R^5$;

wherein a + b equals from 11 to 14 and b is an integer from 1 to 14, wherein R^5 is as set forth above, or a pharmaceutically acceptable salt thereof. The invention also provides preserved compositions comprising the product of the method.

Of course, all embodiments of the invention can be modified in accordance with any of the applicable preferred or alternative embodiments.

Definitions

The terms or phrases listed below shall have the following meaning:

• **antimicrobial activity** encompasses killing microbes, inhibiting the reproduction of microbes, and inhibiting the adherence of microbes to animal tissue.

• An **antimicrobially effective amount** of a compound of formulas (1)-(5) is an amount effective to either (a) reduce the symptoms of a microbial disease sought to be treated, (b) induce a pharmacological change relevant to treating a microbial disease sought to be treated, (c) inhibit or prevent infection or re-infection by a microbial agent, or (d) reduce the adherence of a microbial agent to a tissue. Since the compositions of the inventions can accentuate the activity compounds of formulas (1)-(5), an antimicrobially effective amount is an amount effective when delivered in the relevant composition. For wound treatment, in one aspect, an effective amount includes an amount which, if regularly applied, prevents the occurrence of infection.

• a **bioactive agent** is an agent that is useful for diagnosing or imaging or that can act on a cell, organ or organism, including but not limited to drugs (pharmaceuticals) to create a change in the functioning of the cell, organ or organism. Such agents can include but are not limited to nucleic acids, polynucleotides, antibacterial agents, antiviral agents, antifungal agents, anti-parasitic agents, tumoricidal or anti-cancer agents, proteins, toxins, enzymes, hormones, neurotransmitters, glycoproteins, immunoglobulins, immunomodulators, dyes, radiolabels, radio-opaque compounds, fluorescent compounds, polysaccharides, cell receptor binding molecules, anti-inflammatories, anti-glaucomic agents, mydriatic compounds and local anesthetics.

• an **excipient** is an inert substance used as a diluent or carrier for a pharmaceutically active substance.

• an **ionizable group** is a moiety that is predominantly in an ionized form at physiological pH.

• an **isolated compound** is a compound having at least about 60% wt/wt purity.

• a microbe is a bacteria, mycoplasma, yeast or fungi, virus or parasite (such as a malaria parasite).

• a microbe adherence inhibiting effective amount of a compound is an amount that causes a reduction in the portion of a defined inoculum of a microbe that adheres to a tissue to which the microbe normally adheres.

• oral administration includes any administration into the gastrointestinal tract, including rectal administration.

• a substantially purified compound shall be one that has a purity of at least about 85% wt/wt.

• topical administration includes administration to the tissues that form a barrier between the external environment and the internal organs of an animal, including without limitation administration to the skin, gums, buccal tissue, nasal and sinus tissue, ocular tissue, intraurethral tissue, rectal tissue or intravaginal tissue.

• a transdermal transport effective amount of a compound shall be an amount of the compound that leads to an increase in the amount of a topically applied biological agent that reaches the blood stream of an animal to which the biological agent was applied.

Brief Description of the Drawings

Figure 1 shows a schematic for a synthesis of C16:1Δ6.

Figure 2 shows a timecourse for the adherence of *C. albicans* to stratum corneum.

Figure 3 shows the adherence activity of various Candida isolates.

Figure 4 shows the adherence of *C. albicans* to stratum corneum, stratum corneum with added skin lipid, and lipid depleted stratum corneum.

Figure 5 shows the effect of adding various fractions of skin lipid to stratum corneum discs.

Figure 6 shows the effect of adding C16:1Δ6 and C16:1Δ9 to stratum corneum discs.

Figure 7 shows the bacteriocidal activity C16:1Δ6 against *S. aureus*.

Detailed Description

In preferred embodiments, compositions containing a fatty acid or derivative of formulas (1)-(5) comprise a substantially purified fatty acid or derivative of formulas (1)-(5). Preferably, the fatty acid or derivative shall comprise at least about 0.01% wt/wt of the composition. More preferably the fatty acid or derivative will comprise at least about 0.05% of the composition, still more preferably at least about 0.1%.

In preferred embodiments of the pharmaceutical composition embodiments of the invention, the composition includes a compound of formulas (1)-(5) that lacks an ionizable group in the R^1, R^2, R^3, R^4, or R^5 group, but which is convertible to an ionizable group by either a hydrolysis or oxidation reaction. Preferably, the compound is convertible by a hydrolysis reaction. Also preferably, the compound is converted to an acid, or a salt thereof. More preferably, the convertible compound is an ester of an acid.

Preferred fatty acids or derivatives for use in the invention will have a primary dermal irritation index of less than about 2 PDII grade when 0.2 mg of fatty acid or derivative is applied to a 1.0 cm^2 portion of a rabbit ear, more preferably less than about 1 PDII grade under these conditions. A suitable non-irritating diluent for use in comparative irritation measurements comprises a gel ointment of 2.5% hydroxypropyl methylcellulose in a water/alcohol mixture comprising from 5 to 75% of an alcohol such as ethanol or propanol. The gel can contain other suitable exipients or carriers.

For topical creams or ointments, preferably the compounds of formulas (1) - (5) will comprise between about 0.1% and 5.0% (w/v) of the composition, more preferably between about 0.3% and 3.0%, still more preferably between about 0.4% and 1.0%.

The compositions of the invention can be administered to an animal such as a human in need of protection from microorganisms or of treatment for an infection. Typical modes of administration will include topical, oral, parenteral or pulmonary (by use of an aerosol) administration. The compositions can be administered alone, or they can be combined with a pharmaceutically-acceptable excipient according to standard pharmaceutical practice. For pulmonary administration, excipients will be selected to be appropriate to allow the formation of an aerosol. For the oral mode of administration, the fatty acids and derivatives of the invention are used in the form of tablets, capsules, lozenges, chewing gum, troches, powders, syrups, elixirs, aqueous solutions and suspensions, and the like. In the case of tablets, carriers that is used include lactose, sodium citrate and salts of phosphoric acid. Various disintegrants such as starch, and lubricating agents such as magnesium stearate and talc, are commonly used in tablets. For oral administration in capsule form, useful diluents are lactose and high molecular weight polyethylene glycols. If desired, certain sweetening and/or flavoring agents are added. For parenteral administration, sterile solutions of the fatty acids and derivatives of the invention are usually prepared, and the pHs of the solutions are suitably adjusted and buffered. For ocular administration, ointments or droppable liquids may be delivered by ocular delivery systems known to the art such as applicators or eye droppers. Such compositions can include mucomimetics such as hyaluronic acid, chondroitin sulfate, hydroxypropyl methylcellulose (e.g., available from Dow Chemical, Midlands, MI) or polyvinyl alcohol, preservatives such as sorbic acid, EDTA or benzylchronium chloride, and the usual quantities of diluents and/or carriers. For pulmonary administration, diluents and/or carriers will be selected to be appropriate to allow the formation of an aerosol. Typical excipients or carriers used for topical creams or ointments include hydrophilic gums such as gelatin, sodium

carboxymethylcellulose, pectin, hydroxypropylmethylcellulose and alginates.

Suppository forms of the fatty acids and derivatives of the invention are useful for vaginal, urethral and rectal administrations. Such suppositories will generally be constructed of a mixture of substances that is solid at room temperature but melts at body temperature. The substances commonly used to create such vehicles include theobroma oil, glycerinated gelatin, hydrogenated vegetable oils, mixtures of polyethylene glycols of various molecular weighty and fatty acid esters of polyethylene glycol. *See*, Remington's Pharmaceutical Sciences, 16th Ed., Mack Publishing, Easton, PA, 1980, pp. 1530-1533 for further discussion of suppository dosage forms. Analogous gels or creams can be used for vaginal, urethral and rectal administrations.

Numerous administration vehicles will be apparent to those of ordinary skill in the art, including without limitation slow release formulations, liposomal formulations and polymeric matrices.

The compositions of the invention are preferably administered via devices for dwelling on the skin such as wound dressings, ostomy devices, IV tapes, and the like. For wound dressings, the wound dressing will preferably be designed to administer to the wound or adjacent skin an antimicrobial effective amount of a compound of formulas (1)-(5). Examples of suitable dressings are found, for example, in U.S. Patent Nos. 4,909,243, 4,538,603, 5,244,457 and 5,308,313.

In one embodiment, the compositions of the invention are formulated in hydrocolloid gels. Such gels typically include water soluble hydrocolloids such as pectin, gelatin, guar gum, locust bean gum, gum karaya, and mixtures thereof. Pectin and gelatin are preferred.

For treating or preventing acne, the invention is believed to function by limiting the growth of bacteria or limiting the skin adherence

of bacteria, where the bacteria is associated with the formation of acne lesions. One such species of bacteria involved in the formation of acne lesions is *Propionibacterium acnes*.

The invention, particularly the embodiments comprising a component in the composition of the invention, also provides for the treatment or prevention of gram negative infections or infections by microbes that have acquired drug resistance, such as methacillin resistant *Staphylococcus aureus* ("MRSA"). Preferred gram negative targets for treatment or prevention include *Pseudomonas* infections, such as those caused by *P. acidovorans*, *P. aeruginosa*, *P. cepacia*, *P. diminuta*, *P. fluorescens*, *P. maltophilia*, *P. pseudoalcaligenes*, *P. pseudomallei*, *P. pyocyanea* and *P. stutzeri*, *Escherichia* infections such as those caused by *E. coli*, *Serratia* infections such as those caused by *S. marcescens* and *Klebsiella* infections. Preferred parasite targets for treatment or prevention include *Echinococcus* infections. Preferred fungal treatment or prevention targets include for example *Candida* fungi such as *C. Albicans*, *Pityrosporum* fungi such as *P. ovale* or *P. orbiculore*, *Trichosporon* funge such as *T. rubrumi*, *T. tonurans* or *T. interigitale*, *Microsporum* fungi such as *M. canis* or *M. audouinii*, *Aspergillus* fungi, *Pyrenochaeta* fungi, *Scopulariopsis* fungi such as *S. brevicaulis* and *Acrononium* fungi. All of the above-recited fungi are classified as yeast.

For inhibiting the adherence of microbes, the preferred tissues for treatment with the anti-adherence compounds of the invention are skin, stomach, urinary tract, and intravaginal tissues. Skin is most preferred.

For lipid-replenishing ointments, the ointment will generally comprise no more than about 5 % (wt/wt) of a compound according to formula (5). Preferably, the ointment will comprise no more than about 1% of a compound according to formula (5), more preferably, no more than about 0.5%.

The transdermal administration form of the invention can be used to administer biological agents across any of the several barrier tissues separating the outside environment from the internal organs, including without limitation the skin, gums, buccal tissue, rectal tissue, nasal tissue and sinus membranes. In the transdermal transport method, the compounds of formulas (1)-(5) can be applied to the subject before, concurrently with, or after the application of the biological agent that is to be transdermally administered.

The animals to be treated with the various compositions of the invention are preferably mammals, particularly humans.

The invention encompasses using a cream or ointment according to the invention in conjunction with ostomy products. For instance, a cream of the invention can be used to coat the face plates or seals on ostomy products. See, for example, U.S. Patent Nos. 4,465,486, 4,490,145, 4,460,363 and 4,826,493.

The invention further encompasses using the fatty acids or derivatives as additives to antimicrobial compositions that further include other active agents such as antimicrobials (such as antibiotics, including bacitracin), antifungal agents (such as miconazole and triconazole), spermicidals (such as nonoxynol-9), and the like.

The compositions of the invention are preferably formulated to have pH less than about 7, to minimize irritation to the treatment subject.

The component of the invention is preferably a (C2-C7) alkyl alcohol or a poly(alkylene oxide) wherein the alkylene moieties are C2 to C4, or mixtures of alcohol and poly(alkylene oxide). Preferably, the alcohol is C2-C4, more preferably C3, still more preferably isopropanol. The alkylene of the poly(alkylene oxide) is preferably C2-C3. The poly(alkylene oxide) can be a copolymer of differing alkylene subunits.

Isopropyl alcohol is a preferred alcoholic component used with the invention. Preferred alkyleneoxide polymers include those with ethyleneoxide or propyleneoxide polymer building blocks such as polyethylene glycol and polypropylene glycol. The average molecular weight of the polymer is preferably between about 400 and about 1,000, more preferably between about 400 and about 800. Various weight-range polymer compositions can be mixed to obtain the consistency desired for a particular composition. Compositions of fatty acid-related compounds according to formulas (1)-(5) and an alcoholic component have the following preferred compositions:

	% of composition - preferred	% of composition - more preferred
fatty acid-related compound	about 0.1% to about 10%	about 0.1% to about 0.5%
alcohol	about 1% to about 15%	about 5% to about 10%

Compositions of fatty acid-related compounds according to formulas (1)-(5) and a polymeric component have the following preferred compositions:

	% of composition - preferred	% of composition - more preferred
fatty acid-related compound	about 0.1% to about 10%	about 0.1% to about 0.5%
component polymer	about 2.0% to about 99%	about 6.0% to about 99%

Compositions of fatty acid-related compounds according to formulas (1)-(5), an alcoholic component and a polymeric component have the following preferred compositions:

	% of composition - preferred	% of composition - more preferred
fatty acid-related compound	about 0.1% to about 10%	about 0.1% to about 1.0%
alcohol	about 1% to about 15%	about 5% to about 10%
component polymer	about 20% to about 98.9%	about 60% to about 99%

The syntheses of C16:1Δ6 and C18:1Δ8 were conducted by the same method. The C16:1Δ6 synthesis is outlined in Figure 1. As a first step in synthesizing C16:1Δ6, 1-chloro-5-pentadecyne was synthesized by reacting 1-chloro-4-bromo-butane with undecyne. Second, the 1-chloro-5-pentadecyne intermediate produced by the first reaction was reacted with potassium cyanide to replace the chloro radical with a cyano radical. Third, this nitrile intermediate was reacted with a methanolic mixture of HCl and sulfuric acid to create methyl-6-hexadecynoate. Fourth, the methyl ester produced by the third reaction was hydrolyzed to create 6-hexadecynoic acid. Fifth, the acid was reduced by catalytic hydrogenation using a palladium on barium sulfate catalyst to create 6(Z)-hexadecanoic acid (C16:1Δ6) (having cis configuration at the double bond).

For the first synthesis step, the solvent conditions must be sufficiently basic to favor proton abstraction from decyne. A mixture of ammonia and $NaNH_2$ in tetrahydrofuran is a preferred reaction medium. Temperatures that are low enough to maintain the ammonia in liquid form are preferred. The undecyne (for example, from Lancaster Co., Windham, NH) is added to the basic solvent incrementally. Then, the akylhalide (or comparable alkyl compound that is α,ω-di-substituted with an appropriate leaving group) is added incrementally to the reaction. These incremental additions of reactants prevent over rigorous reaction. For a description of this type of reactiion, see, Sprecher, "The Organic

Synthesis of Unsaturated Fatty Acids," *Prog. Chem. Fats other Lipids* 15: 219-254, 1978.

For the second synthesis step, the solvent is preferably anhydrous and aprotic. Preferably, the solvent is also polar. Dimethylsulfoxide ("DMSO") is a preferred solvent. Potassium cyanide is the preferred source of cyanide anion.

For the third reaction, the nitrile functionality is converted to a carboxylate functionality. This can be accomplished by a hydrolytic reaction, such as using acidic or basic hydrolytic conditions, as will be recognized by those of ordinary skill. Preferably, the hydrolysis is accomplished in an acidic, alcoholic solvent. Particularly preferred is a mixture of methanolic HCl and concentrated H_2SO_4. These preferred conditions will generate the alcohol ester derivative of the nitrile.

If the third reaction generates an ester, the fourth reaction comprises a hydrolysis reaction. Conditions effective to hydrolyze an ester are well known to those of ordinary skill. One such method is base-catalyzed hydrolysis, for instance using 0.5% wt/v NaOH in a mixture of 100 ml H_2O and 300 ml methanol (which is added to increase the solubility of the ester). Generally, ester hydrolyses proceed at a modest temperature, such as room temperature.

For the final reaction, the catalyst is preferably palladium on barium sulfate (for example, 5% palladium on sulfate from Aldrich Chemical Co., Milwaukee, WI) The solvent is selected to "poison" the catalyst to adjust its reactivity. Pyridine and quinoline are appropriate solvent components for so adjusting catalyst reactivity. Anhydous pyridine is the preferred solvent. It will be apparent that these synthetic methods can be modified by substituting the starting materials to make other acids within formulas (1) - (5).

The amine compounds of formulas (1) - (5) can be synthesized, for example by reducing the corresponding amides with a hydride as described, for example, in Section 4.1 of Carey and

Sundberg, Advanced Organic Chemistry, Part B, Plenum Press, New York, 1977 or by Hoffmann rearrangement to remove the carbonyl moiety, as described in Wallis and Lane, *Org. React.* 3: 267, 1946.

The amide compounds of formulas (1) - (5) can be synthesized, for instance, by conducting a dehydration reaction between the corresponding fatty acid and $NHR^{14}R^{15}$. Such a reaction can be conducted by first forming an activated derivative of the acid such as an anhydride or an N-hydroxysuccinimide ester. Alternately, the dehydration reaction can be conducted using a carbodiimide compound to form a reactive intermediate with the acid moiety. Alternatively, the acid synthesized as described above can be converted to the acid halide, for instance by reaction with a halogen gas in the presence of phosphorus or with a halogen-substituted phosphorus compound such as phosphorus trichloride, and subsequently reacted with the amine moiety. In converting an acid to the acid halide, care should be taken to limit α-halogenation.

The ester compounds of formulas (1) - (5) wherein R^1, R^2, R^3, R^4, or R^5 is an ester with glycerol or monoglyceride or can be formed using the same dehydration reactions described above.

The alcohol compounds of formulas (1) - (5) wherein R^1, R^2 R^3, R^4, or R^5 is CH_2OH can be synthesized by reducing the corresponding esters with a hydride as described, for example, in Section 4.1 of Carey and Sundberg, Advanced Organic Chemistry, Part B, Plenum Press, New York, 1977.

The following examples further illustrate the present invention, but of course, should not be construed as in any way limiting its scope.

Example 1 - Synthesis of 6(Z)-Hexadecanoic Acid

1-bromo-4-chlorobutane, tetrahydrofuran ("THF") and sodium amide were from Aldrich (Milwaukee, WI); undecyne was from Lancaster (Windham, NH) and anhydrous ammonia was from MG

Industries (Messer Griesheim Industries, Malvern, PA). (THF may require redistillation to limit the peroxide content.) All glassware was oven dried and cooled under dry N_2. A stir bar and 200 ml dry THF were placed into a 500 ml three necked flask fitted with a cold trap, a nitrogen inlet, and a mineral oil bubbler. The flask was cooled to -78°C in a solid carbon dioxide (dry ice)-acetone bath. A mixture of dry ice and acetone was also used to cool the cold trap. Ammonia (100 ml) was condensed in the cold trap. The ammonia was then condensed into the flask and 12 g (0.3 mol) of $NaNH_2$ was added under N_2, creating a milky white mixture. To this mixture 38 ml (30 g, 0.2 mol) undecyne was added dropwise over a 10 minute period. The mixture then became thick and viscous. Additional THF was added (70 ml) to reduce the viscosity and allow continued stirring. After stirring for 30 minutes, 25 g (0.145 mol) of 1-bromo-4-chlorobutane was added dropwise to the mixture over a 1 hour period. This mixture was stirred for an additional 3 hours at -78°C, and then allowed to warm and reflux for an additional 2 hours. The reaction was monitored by silica gel TLC developed in 100% petroleum ether. In this TLC system undecyne migrates with the solvent front, bromochlorobutane migrates with an RF of 0.60, although it stains only lightly with iodine or charring. The product has an RF of 0.66. No other spots were observed. The reaction mixture was then allowed to gradually warm to 25°C and stirred under positive nitrogen pressure for 15 hours. After this, the reaction was quenched by the addition of 20 g NH_4Cl. The reaction mixture was then poured into 200 ml cold deionized ("DI") water and 200 ml hexane was added to the resulting mixture. The organic layer was separated, and the aqueous layer was extracted twice more with 100 ml hexane. The combined organic layers were washed (1) with 200 ml DI water and (2) with 200 ml brine (i.e., an aqueous solution saturated with sodium chloride at 25°C). The washed organic layer was dried over Na_2SO_4 and filtered. The solvent was removed under vacuum, and the product isolated by

vacuum distillation. At 5 mm Hg the excess undecyne distilled off at a temperature between 46-54°C. The pure 1-chloro-4-pentadecyne product distilled at a temperature between 138-144°C. The yield was 19.4 g (0.080 mol) purified 1-chloro-4-pentadecyne or 55%.

In a 1 L flask fitted with a thermometer and a stirring bar, 20 g (.031 mol) KCN (Fisher) was suspended in 400 ml anhydrous DMSO (Aldrich). (Caution, KCN in DMSO is extremely toxic and readily absorbed through skin). 1-chloro-4-pentadecyne (19.4 g) was added and the mixture gradually heated to 98°C until reaction completion as monitored by silica gel TLC. (Product RF is 0.43 in 100% pet. ether). When the reaction was complete (after about 3 hours), 200 ml DI water and 200 ml hexane were added. The organic layer was separated, and the aqueous layer extracted twice more with 150 ml portions of hexane. The combined organic layers were washed twice with 100 ml portions of DI water and once with 100 mL brine. The washed organic layer was dried over Na_2SO_4 and the solvent removed *in vacuo*. The yield was 18.1 g (94%) of straw-colored oil containing 1-chloro-4-pentadecyne that was used without further purification.

The straw-colored oil was added to a 1 L flask, fitted with a condenser and drying tube, containing 400 ml of 2.0 M methanolic HCl. Concentrated H_2SO_4 (10 ml) was added and the mixture was brought to reflux with stirring. The solution was refluxed until the reaction was complete as monitored by silica gel TLC. (Product RF is 0.81 in 70:30 pet. ether ("PE"): ethyl ether ("EE"); starting material RF is 0.65). Additional concentrated H_2SO_4 (10 ml) was added daily. The reaction produced several side products. Upon completion (after five days), 200 ml DI water were added, and the mixture extracted with three 200 ml portions of hexane. The combined organic layers were washed with 200 ml water, 200 ml 5% (w/v) aqueous sodium bicarbonate, and 200 ml brine. The washed organic layer was then dried over Na_2SO_4, filtered and the solvent evaporated *in vacuo*. The

resulting crude reddish oil was purified by flash column chromatography (5 x 4 cm, Silico-60, from EM Science, Cherry Hill, NJ) with elution by 85:15 PE:EE. 12.6 g (57% yield) of methyl-6-hexadecynoate was recovered as a colorless oil.

The methyl ester product was converted to the corresponding acid by refluxing it in a solution of 20 g NaOH dissolved in 300 ml methanol and 100 ml DI water for 3 hours or until completion as measured by silica gel TLC (RF for free acid is 0.35 in 70:30:2 PE:EE:acetic acid). Upon reaction completion about ½ of the methanol was removed *in vacuo*. The mixture was then acidified with concentrated HCl to a pH of under 3. This mixture was then extracted with 3 x 150 ml portions of hexane. The combined organic extract was washed with 150 ml DI water and 150 ml brine, and dried over Na_2SO_4. The solvent was removed *in vacuo* to give a quantitative yield of 6-hexadecynoic acid as an oil (Optionally, the product can be recrystallized at 20°C from hexane to yield white crystals (M.P. 37°C). However, the recrystallization results in only 70% recovery and may be omitted).

The 6-hexadecynoic acid was added to a 1L flask with 300 ml anhydrous pyridine (Aldrich) along with 1.0 g of 5% Pd on $BaSO_4$ catalyst (oxidized from) and sealed with a rubber septum. The catalyst is available from Aldrich Chemical Co., Milwaukee, WI. The flask (which was vented with a needle vent) was first purged with nitrogen then hydrogen through a needle cannula. The flask was kept under 2 lbs. positive H_2 pressure, and the reaction monitored by the uptake of hydrogen. When H_2 uptake had ceased the completion of the reaction was analyzed by gas chromatography (SP2330 column, Supelco, Inc., Bellefonte, PA) of an esterified sample. Hydrogen uptake had essentially ceased after 3 hours. However, the reaction was continued overnight. Then, the flask was purged with nitrogen. After which, the catalyst was removed by filtration. The pyridine was then removed under

vacuum. The final purification was by column chromatography with a 5 x 50 cm Silica-60 column. The column was equilibrated with 90:10 PE:EE. The crude product was applied to the column following acidification with 1 ml acetic acid. The column was developed step-wise with 500 ml 90:10 PE:EE, 500 ml 80:20 PE:EE, and 500 ml 70:30 PE:EE. The fractions containing pure 6-hexadecanoic acid (by TLC) were pooled and evaporated. A final yield of 7.6 g pure 6(Z)-hexadecanoic acid was isolated with an additional 5 g of impure product (90% desired product) retained for repurification.

The purified product was analyzed by silica gel TLC run in 70:30:2 PE:EE:acetic acid using palmitoleic acid as a reference standard. TLC indicated greater than 99% purity. Analytical nuclear magnetic resonance ("NMR") spectroscopy showed a multiplet pattern indicative of a predominately cis configuration of the double bond.

Example 2 - Synthesis of 8(Z)-Hexadecanoic Acid .

The same procedures that were outlined above were used except 1-chloro-6-bromohexane was substituted for 1-chloro-4-bromobutane. In the first reaction, 20.4 g of 1-chloro-6-heptadecyne was recovered. The 1-chloro-6-heptadecyne product had a boiling point at 5 mm Hg of 152-157°C. The second reaction produced about 18 g of 1-cyano-6-heptadecyne. The fourth reaction produced 18.1 g or 6(Z)-octadecynoic acid. In the final reductive reaction, the catalyst was replaced to allow the reduction to go to completion. The purified yield of 8(Z)-octadecenoic acid was 9.6 g. TLC indicated greater than 99% purity. NMR indicated a cis configuration of the carbon-carbon double bond. Gas chromatography, using the methyl ester derivative, indicated that 96.5% of the product had cis configuration. Oleic acid (9Z) and elaidic acid (9E) were used as gas chromatography standards.

Example 3 - Adherence Assay

To obtain stratum corneum tissue, immediately after slaughter pigs were shaved and a heated aluminum cylinder (65°C) was

applied to the skin for 30 seconds. A corresponding circular section of epidermis was then peeled from the underlying connective tissue and, if not immediately processed further, stored at -20°C until use. Ten such circular sheets of epidermis were placed in 100 ml of 0.5% trypsin (Type III, Sigma Chemical Co., St. Louis, MO) in 20 mM phosphate buffered isotonic saline ("PBS") at pH 7.4. The epidermis was incubated with the trypsin overnight at 4°C. The digested tissue was then rinsed with distilled water and placed in a fresh trypsin solution and incubated at 37°C for two hours with gentle agitation. The resulting stratum corneum was rinsed with distilled water and blotted dry. One cm discs of stratum corneum were cut for use in the experiments outlined below. The stratum corneum discs were sterilized by autoclaving before storage and use.

1 cm discs of stratum corneum were suspended in 1 ml of a suspension of *C. Albicans* fungi containing 5 x 1.0^5 cells per ml. In a prior study, the yeast suspension was incubated with the stratum corneum with gentile agitation for from 0 to 3 hours. These initial studies indicated that an incubation time of 2 hours was suitable. Accordingly, the suspensions were incubated for 2 hours with gentle agitation provided by a rotary shaker. The discs were then transferred to clean test tubes and rinsed three times with 5 ml portions of PBS. After rinsing, the discs were placed on glass slides and stained with periodic acid-Schiff reagent. The slides were examined through a microscope and the stained, attached organisms in ten randomly selected fields were counted, and the number averaged.

A time course for the adherence of *Candida albicans* to skin is shown in Fig. 2.

Example 4 - Adherence of Various Candida Strains

Three strains of human pathogenic candida fungi (HPI-1, HPI-2 and HPI-3) (isolated from symptomatic patients), one candidal strain of human commensurate (i.e., symbiotic, non-pathogenic) fungi

(HCI-1) (isolated from asymptomatic individual), and a laboratory, non-pathogenic strain, *Candida parasitosis* were tested for their ability to adhere to stratum corneum using the method outlined in Example 3. The results, measured after 2 hours incubation of the fungus with stratum corneum, were as illustrated in Fig. 3.

Example 5 - Effects of Lipid Removal and Addition

The effect of removing or adding skin lipids to skin on the ability of *C. albicans* to adhere to skin was measured. Stratum corneum discs were lipid-deleted by successive extractions with chloroform:methanol solutions having the following ratios: 2:1, 1:1 and 1:2 (v:v). Each extraction was conducted at room temperature for two hours with gentle agitation. The discs were air dried overnight.

For use in adding additional lipid to skin, a ethanol extract of surface skin lipid was prepared as described in Wertz et al., *J. Invest. Dermatol.* 84: 410-412, 1985. Briefly, human volunteers positioned the wrist portions of their arms over a stainless steel basin, with the hand and the rest of the arm angled up and away from the basin. A 250 ml portion of 95% ethanol was slowly poured over each wrist and the lipids that extracted into the ethanol were recovered by evaporating the solvent.

To add skin lipids to stratum corneum discs, the total lipid fraction so isolated was dissolved in hexane: isopropanol, 3:2, at a concentration of 1 mg/ml and 50 μl portions (0.05 mg lipid) were applied to stratum corneum discs. The solvent was then evaporated from the discs. Samples of normal stratum corneum ("sc"), lipid-depleted stratum corneum ("-lipid"), and stratum corneum containing additional lipid ("+lipid") were tested for the ability of *C. albicans* to adhere thereto, as described in Example 3. The results are shown in Fig. 4.

Example 6 - Addition of Specific Skin Lipid Fractions to Stratum corneum

A part of the skin lipid extract described in Example 5 was fractionated on a preparative (0.25 mm thick) silica gel plate (Adsorbosil plus one®, Alltech Associates, Deerfield, IL) developed with hexane:ethyl ether:acetic acid, 70:30:1 (v:v). Lipid fractions were located using a scanning photodensitometer set at 210 nm. The lipid-containing regions of the plate were scrapped and the lipids eluted using chloroform:methanol:water, 50:50:1. By this method, fractions containing (1) squalene ("SQ"), (2) wax esters and cholesterol esters ("WE/CE"), (3) triglycerides ("TG"), (4) fatty acids ("FA"), (5) cholesterol ("CH") and (6) a polar fraction made up primarily of ceramides and cholesterol sulfate ("POLAR") were isolated.

Using the same methodology outlined in Example 5, the specific lipid fractions described above were added to stratum corneum and the adherence of *C. albicans* was measured. The results are shown in Figure 5.

Example 7 - Inhibition of *C. albicans* adherence by C16:1$_{\Delta}$6 and C16:1$_{\Delta}$9

Using the methodology of Example 5, 0.1, 1.0 and 10.0 mg of C16:1$_{\Delta}$6 or C16:1$_{\Delta}$9 was applied to 1 cm stratum corneum discs and the effect of adherence by *C. albicans* was measured. The results are shown in Figure 6.

Example 8 - Effect of Lipid on Bacteria

A preparation of skin surface lipid prepared by extracting human hair clippings with chloroform:methanol, 2:1, for 2 hours at room temperature. Following the extraction, the lipids were recovered by evaporating the extraction solvent. The lipids of hair clippings are believed to reflect the composition of sebum free of lipid derived from sweat gland secretions. The skin lipids or other lipid preparations were suspended or dissolved in beef heart infusion broth (BHIB, Difco, Livonia, MI) by sonication (taking care not to overheat the broth).

Bacteria (10^8 colony forming units ("CFU") per ml) were suspended in beef heart infusion broth, with or without lipid, and incubated at 37°C with agitation. After various intervals, samples were taken from the incubations, diluted and plated on BHIB agar plates to determine the number of colony forming units ("CFUs") present. The results were measured by the number of CFUs versus time.

Using this same methodology, the lipid extracted from skin has been shown to be bacteriocidal towards *S. aureus*, *Streptococcus salivarius*, *Eichinella corodens* and *Fusobacterium nucleatum* (a gram negative anaerobic bacteria involved in gum disease), and to be bacteriostatic (i.e., to prevent growth) against *E. fascalis*. Activity was not detected against *P. aeruginosa*.

Example 9 - Inhibitory Activity of Specific Lipids

The procedures of Example 8 were repeated using specific fatty acids and testing against both *S. aureus* and *S. salivarius*. In this experiment, the optical density of the cultures was used to indicate the relative numbers of bacteria present, rather than measuring CFU values. The results, in terms of minimum inhibitory concentrations ("MICs") were as follows:

Fatty Acid	MIC Against *S. aureus* (mg/ml)	MIC Against *S. saliv.* (mg/ml)
C16:1Δ6	0.03	0.05
C18:1Δ8	n.d.	1.0
C16:1Δ9	0.03	0.05

The underlying data for C16:1Δ6 against *S. aureus* are shown in Figure 7.

Example 10 - Effect of Lipid and Alcohol on Methicillin-Resistant *S. aureus*

The effects of palmitoleic acid and ethanol (15%) on methicillin-resistant *S. Aureus* (MRSA) were tested using the

methodology of Example 8. The results, shown as the log of the colony forming units (cfus) measured by plating after 5 minutes exposure, were as follows for a first MRSA strain:

Treatment	Log (cfu/ml)
Control	7.8
Palmitoleic acid (100 μg/ml)	6.8
Ethanol (15%)	7.6
Palmitoleic Acid + ethanol	0

The results, shown as the log of the colony forming units (cfus) measured by plating after 5 minutes exposure, were as follows for a second MRSA strain:

Treatment	Log (cfu/ml)
Control	7.4
Palmitoleic acid (100 μg/ml)	7.2
Ethanol (15%)	7.2
Palmitoleic Acid + ethanol	4.1

Example 11 - The Primary Dermal Irritation Index for Specific Compositions

The primary dermal irritation index ("PDII") of various compositions, each formulated in 2.5% hydroxypropylmethylcellulose in 3:1 ethanol:water, was measured using an albino rabbit, single insult patch test. Two test areas per rabbit were prepared by shaving both areas, and abrading one of the areas. Similarly, two matching control areas were prepared on each animal. The test material, 0.5 ml each application, was applied to 2.5 x 2.5 cm gauze patches, and held

against the test area with an impervious Vetrap brand bandage (3M, St. Paul, MN). The patches were held in place for 24 h, at which time the treatment sites were wiped clean. The evaluations were based on observations at this 24 h timepoint and on observations at the 72 h timepoint. Scoring was as set forth in Draize, "Dermal Toxicity," Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics - Dermal Toxicity, pp. 46-59, Association of Food and Drug Officials of the U.S., Topeka, Kansas, 1965. The results for individual fatty acids were as follows:

Fatty Acid	Concentration (% w/w)	PDII
C14:1Δ9	2 10	0.55 1.75
C16:1Δ6	2 5 10	0 0.25 1.50
C16:1Δ9	2 5 10	0 0 0
C18:1Δ9	2	0.65
C:18:2Δ9, 12	2 10	0.42 2.0

Example 12 - A Further Test for Antimicrobial Activity

A drop of bacteria suspended in PBS, containing 3×10^5 bacteria, was deposited on a sterilized Whatman filter No. 4 (4.4 cm^2). A gel composition containing a prospective antimicrobial agent was layered over the filter paper, with care taken to avoid air pockets formed between the gel and the filter. This amount of bacteria is in excess of the amount (1×10^5) considered to constitute an infection when present in one gram of tissue. The filters with bacteria and gel compositions were maintained at room temperature for 20 minutes. The filters were then placed in 25 ml of Trypticase Soy Broth Z-49 medium (available

from GIBCO (Grand Island, NY) and incubated in a shaker incubator for 24 hours at 38.6°C at 132 rpm. Positive results were scored when bacteria could not be observed microscopically in the culture medium.

Example 13 - Various Formulations

The following formulations, described in weight percentages, were prepared:

Composition:	1	2	3	4	5
C16:1Δ6	0.38%	0.39%	0.28%	0.31%	0.31%
Ethanol		11.0%		10.2%	5.5%
PEG[1]	99.62%		83.7%		
Glycerol		88.6%		87.3%	93.7%
Propylene glycol			16.0%		
Gelatin[2]				2.2%	
Pectin[3]					0.46%

[1]The polyethylene glycol was PEG-400, available from Aldrich Chemical Co., Milwaukee, WI.

[2]The gelatin was obtained from Hormel, Davenport, IA.

[3]The pectin was obtained from Citrus Colloids, Hereford, United Kingdom.

Compositions 1,3 and 4 were transparent liquids. Composition 2 was a liquid when freshly prepared, but subsequently separated into two phases. Compositions 5 and 6 were gels.

Example 14 - Various Formulations

The following formulations, described in weight percentages, were prepared:

Composition:	6	7	8	9	10
C16:1Δ6	0.26%	0.26%	0.28%	0.26%	0.29%
Ethanol	9.6%	9.8%	5.3%	4.7%	
Isopropanol					6.3%
Glycerol	88.4%	91.4%	87.2%	87.2%	91.9%
NaCMC[1]	1.7%	0.94%	1.7%	0.88%	1.6%

[1]The sodium carboxymethylcellulose (NaCMC) was from Aqualon Ltd., Warrington, United Kingdom.

All of the above-described compositions 6-10 are gels.

Example 15 - Test Results

Composition 10 and a corresponding composition 10-CMP, which lacked the fatty acid component, were tested for antimicrobial activity against *E. coli* using the method of Example 12. The following average optical densities were obtained for cultures grown from filters treated as indicated:

Gel Applied	Bacteria Applied	Av. OD
None	None	0.11
None	Yes	0.99
10	Yes	0.03
10	Yes	0.03
10-CMP	Yes	0.48
10-CMP	Yes	0.70
10	None	0.01
10-CMP	None	0.02

While this invention has been described with an emphasis upon preferred embodiments, it will be obvious to those of ordinary skill in the art that variations in the preferred devices and methods may be used and that it is intended that the invention may be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications encompassed within the spirit and scope of the invention as defined by the claims that follow.

What is claimed:

1. An antimicrobial composition comprising

(A) antimicrobial activity enhancing effective amount of a component selected from the group consisting of (C2-C7) alkyl alcohols, poly(alkylene oxide)s wherein the alkylene moieties are C2 to C4, and mixtures thereof; and

(B) a compound as follows:

(5) $CH_3(CH_2)_a-\underset{H}{\overset{|}{C}}=\underset{H}{\overset{|}{C}}-(CH_2)_b-R^5$;

wherein a + b equals from 11 to 14 and b is an integer from 1 to 14, and wherein R^5 is

(a) $CH_2NR^{21}R^{22}$, wherein R^{21} and R^{22} are independently hydrogen or C1 to C6 hydrocarbon,

(b) C(O)-R^{23}, wherein R^{23} is (i) $NR^{24}R^{25}$, wherein R^{24} and R^{25} are independently hydrogen or C1 to C6 hydrocarbon, (ii) OR^{26}, wherein R^{26} is glyceryl or a glyceryl fatty acid ester, wherein said fatty acid is about C15 to about C18, or (iii) a hydroxyl, or

(c) CH_2OH,

or a pharmaceutically acceptable salt thereof.

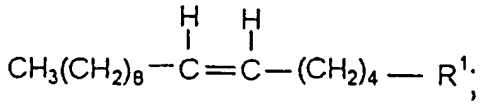
2. The composition of claim 1, wherein the compound of formula (5) is according to one of the following formulas

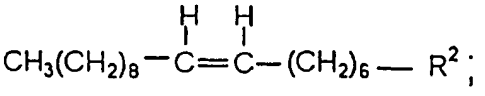
(3) $CH_3(CH_2)_5-\underset{H}{\overset{|}{C}}=\underset{H}{\overset{|}{C}}-(CH_2)_7-R^3$;

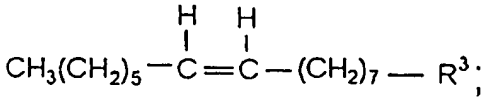
(4) $CH_3(CH_2)_5-\underset{H}{\overset{|}{C}}=\underset{H}{\overset{|}{C}}-(CH_2)_n-R^4$;

and mixtures thereof, wherein n is an integer from 3 to 6 and R^3 and R^4 are independently as set forth for R^5.

3. The composition of claim 1, wherein the compound of formula (5) is according to one of the following formulas

(1)  ;

(2)  ;

(3)  ;

wherein R^1, R^2 and R^3 are independently (d) $CH_2NR^{21}R^{22}$, (e) $C(O)$-R^{13*}, wherein R^{13*} is (i) OR^{26}, or (ii) a hydroxyl, or (f) CH_2OH, or a pharmaceutically acceptable salt thereof.

4. The composition of claim 3, wherein the compound comprises an acid whereby R^1, R^2 or R^3 comprises COOH, or a pharmaceutically acceptable salt thereof.

5. The composition of claim 4, wherein the compound is an acid addition salt and the salt comprises a sodium, ammonium, silver, copper, calcium, barium, zinc or mono-, di- , tri- or quaterniary alkylammonium salt, wherein said alkyl substituents on ammonium are independently C1 to C8.

6. An antimicrobial composition of claim 1, wherein the composition is a toothpaste, mouthwash, shampoo, hair styling composition, skin ointment, make-up composition, anti-oderant or antipersperant.

7. A device for dwelling on the skin comprising the antimicrobial composition of claim 1.

8. A method of treating or preventing an infection of the gastrointestinal tract comprising administering an antimicrobially effective amount of the antimicrobial composition according to claim 1.

9. A method of treating or preventing an acne comprising administering an antimicrobially effective amount of the antimicrobial composition according to claim 1.

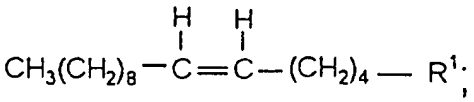
10. A method of preventing the adherence of a microbe to epithelial surfaces comprising administering a microbe adherence reducing effective amount of an antimicrobial composition according to claim 1.

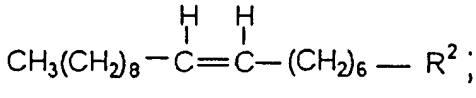
11. A lipid-replenishing skin treatment ointment comprising:

(a) the antimicrobial composition of claim 1, and

(b) a plurality of lipids selected from the lipids normally present on skin.

12. An antimicrobial composition comprising (I) a pharmaceutically acceptable excipient and (II) an isolated compound of formula:

(1)  ;

(2)  ;

wherein R^1 and R^2 are independently

(a) $CH_2NR^{11}R^{12}$, wherein R^{11} and R^{12} are independently hydrogen or C1 to C6 hydrocarbon,

(b) C(O)-R^{13},wherein R^{13} is (i) $NR^{14}R^{15}$, wherein R^{14} and R^{15} are independently hydrogen or C1 to C6 hydrocarbon, (ii) OR^{16} , wherein R^{16} is glyceryl or a glyceryl fatty acid ester, wherein said fatty acid is about C15 to about C18, or (iii) a hydroxyl, or

(c) CH_2OH,

or a pharmaceutically acceptable salt thereof.

13. The composition of claim 12, wherein R^1 and R^2 are independently: $CH_2NR^{11}R^{12}$; C(O)-R^{13*}, wherein R^{13*} is (1) OR^{16} , or (2) a hydroxyl; or CH_2OH, or a pharmaceutically acceptable salt thereof.

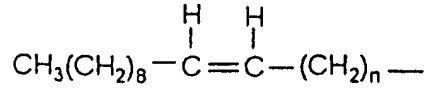
14. The composition of claim 13, wherein the compound is an acid whereby at least one of R^1 or R^2 is C(O)OH, or a pharmaceutically acceptable salt thereof.

15. The composition of claim 14, wherein the compound is an acid addition salt and the salt comprises a sodium, ammonium, silver, copper, calcium, barium, zinc or mono-, di- , tri- or quaterniary alkylammonium salt, wherein said alkyl substituents on ammonium are independently C1 to C8.

16. The composition of claim 12, wherein further comprises an antimicrobial activity enhancing effective amount of a component selected from the group consisting of (C2-C7) alkyl alcohols, poly(alkylene oxide)s wherein the alkylene moieties are C2 to C4, and mixtures thereof.

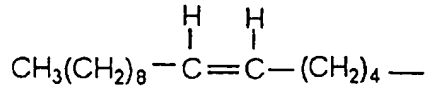
17. A method of treating or preventing infection, the method comprising applying or administering to an animal a composition

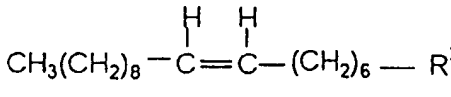
comprising an antimicrobially effective amount of a compound selected from the group consisting of:

(4)  ;

wherein n is an integer from 3 to 6 and R^4 is (a) $CH_2NR^{31}R^{32}$, wherein R^{31} and R^{32} are independently hydrogen or C1 to C6 hydrocarbon, (b) $C(O)\text{-}R^{33}$, wherein R^{33} is (i) $NR^{34}R^{35}$, wherein R^{34} and R^{35} are independently hydrogen or C1 to C6 hydrocarbon, (ii) OR^{36}, wherein R^{36} is glyceryl or a glyceryl fatty acid ester, wherein said fatty acid is about C15 to about C18, or (iii) a hydroxyl, or (c) CH_2OH, or a pharmaceutically acceptable salt of said compound.

18. The method of treating or preventing infection of claim 17, wherein the compound is according to one of the following formulas

(1)  ;

(2)  ;

wherein R^1 and R^2 are independently (d) $CH_2NR^{31}R^{32}$, (e) $C(O)\text{-}R^{13*}$, wherein R^{13*} is (i) OR^{36} or (ii) a hydroxyl, or (f) CH_2OH, or a pharmaceutically acceptable salt thereof.

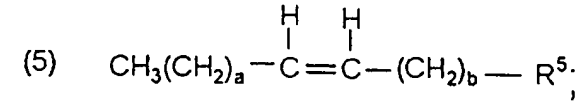
19. The method of treating or preventing infection of claim 18, wherein said compound is an acid whereby R^1 or R^2 is C(O)OH, or a pharmaceutically acceptable salt of the acid.

20. The method of treating or preventing infection of claim 19, wherein the compound is an acid addition salt of a sodium, ammonium, silver, copper, calcium, barium, zinc or mono-, di- , tri- or quaterniary

alkylammonium, wherein said alkyl substituents on ammonium are independently C1 to C8.

21. A method of treating or preventing a microbial infection comprising:

(A) administering or applying is to an epithelial surface of an animal a compound as follows:

(5) 

wherein a + b equals from 11 to 14 and b is an integer from 1 to 14,

wherein R^5 is

(a) $CH_2NR^{21}R^{22}$, wherein R^{21} and R^{22} are independently hydrogen or C1 to C6 hydrocarbon,

(b) C(O)-R^{23}, R^{23} is (i) $NR^{24}R^{25}$, wherein R^{24} and R^{25} are independently hydrogen or C1 to C6 hydrocarbon, (ii) OR^{26}, wherein R^{26} is glyceryl or a glyceryl fatty acid ester, wherein said fatty acid is about C15 to about C18, or (iii) a hydroxyl, or

(c) CH_2OH,

or a pharmaceutically acceptable salt thereof; and

(B) applying or administering to the surface an antimicrobial activity enhancing effective amount of a component selected from the group consisting of (C2-C7) alkyl alcohols, poly(alkylene oxide)s wherein the alkylene moieties are C2 to C4, and mixtures thereof.

22. The method of treating or preventing an infection of claim 21, wherein the compound of formula (5) is of formula (1), (2) or (3) as follows:

(1) $CH_3(CH_2)_8-\overset{H}{\underset{|}{C}}=\overset{H}{\underset{|}{C}}-(CH_2)_4-R^1$;

(2) $CH_3(CH_2)_8-\overset{H}{\underset{|}{C}}=\overset{H}{\underset{|}{C}}-(CH_2)_6-R^2$;

(3) $CH_3(CH_2)_5-\overset{H}{\underset{|}{C}}=\overset{H}{\underset{|}{C}}-(CH_2)_7-R^3$;

wherein R^1, R^2 and R^3 are independently: (d) $CH_2NR^{21}R^{22}$; (e) $C(O)\text{-}R^{13*}$, wherein R^{13*} is (i) OR^{26}, or (ii) a hydroxyl; or (f) CH_2OH, or a pharmaceutically acceptable salt thereof.

23. The method of claim 22, wherein the compound administered or applied comprises an acid whereby R^1, R^2 or R^3 comprises COOH, or a pharmaceutically acceptable salt thereof.

24. The method of treating or preventing infection of claim 23, wherein the compound comprises a sodium, ammonium, silver, copper, calcium, barium, zinc or mono-, di- , tri- or quaterniary alkylammonium acid addition salt, wherein said alkyl substituents on ammonium are independently C1 to C8.

25. A transdermal administration form for a bioactive agent comprising:

(a) the bioactive agent; and

(b) a transdermal transport effective amount of a compound as follows:

(5) $CH_3(CH_2)_a-\overset{H}{\underset{|}{C}}=\overset{H}{\underset{|}{C}}-(CH_2)_b-R^5$;

wherein a + b equals from 11 to 14 and b is an integer from 1 to 14,

wherein R^5 is

(a) $CH_2NR^{21}R^{22}$, wherein R^{21} and R^{22} are independently hydrogen or C1 to C6 hydrocarbon,

(b) C(O)-R^{23}, R^{23} is (i) $NR^{24}R^{25}$, wherein R^{24} and R^{25} are independently hydrogen or C1 to C6 hydrocarbon, (ii) OR^{26}, wherein R^{26} is glyceryl or a glyceryl fatty acid ester, wherein said fatty acid is about C15 to about C18, or (iii) a hydroxyl, or

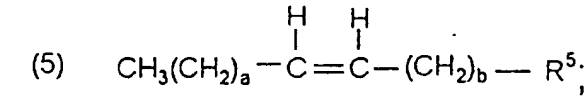
(c) CH_2OH,

or a pharmaceutically acceptable salt thereof.

26. A method of transdermal administration of a bioactive agent comprising the steps of

(a) topically applying a bioactive agent to a site on an animal; and

(b) applying to the site a transdermal transport effective amount of a compound as follows:

(5) 

wherein a + b equals from 11 to 14 and b is an integer from 1 to 14,

wherein R^5 is

(a) $CH_2NR^{21}R^{22}$, wherein R^{21} and R^{22} are independently hydrogen or C1 to C6 hydrocarbon,

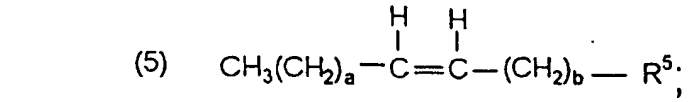
(b) C(O)-R^{23}, R^{23} is (i) $NR^{24}R^{25}$, wherein R^{24} and R^{25} are independently hydrogen or C1 to C6 hydrocarbon, (ii) OR^{26}, wherein R^{26} is glyceryl or a glyceryl fatty acid ester, wherein said fatty acid is about C15 to about C18, or (iii) a hydroxyl, or

(c) CH_2OH,

or a pharmaceutically acceptable salt thereof.

27. The method of claim 26, wherein the applying step (b) occurs prior to or concurrently with applying step (a).

28. A method of preserving a biologically degradable composition comprising contacting the composition with a preservation effective amount of a a compound as follows:

(5) 

wherein a + b equals from 11 to 14 and b is an integer from 1 to 14,

wherein R^5 is

(a) $CH_2NR^{21}R^{22}$, wherein R^{21} and R^{22} are independently hydrogen or C1 to C6 hydrocarbon,

(b) C(O)-R^{23}, R^{23} is (i) $NR^{24}R^{25}$, wherein R^{24} and R^{25} are independently hydrogen or C1 to C6 hydrocarbon, (ii) OR^{26}, wherein R^{26} is glyceryl or a glyceryl fatty acid ester, wherein said fatty acid is about C15 to about C18, or (iii) a hydroxyl, or

(c) CH_2OH.

29. A preserved composition comprising the product of the method of claim 28.

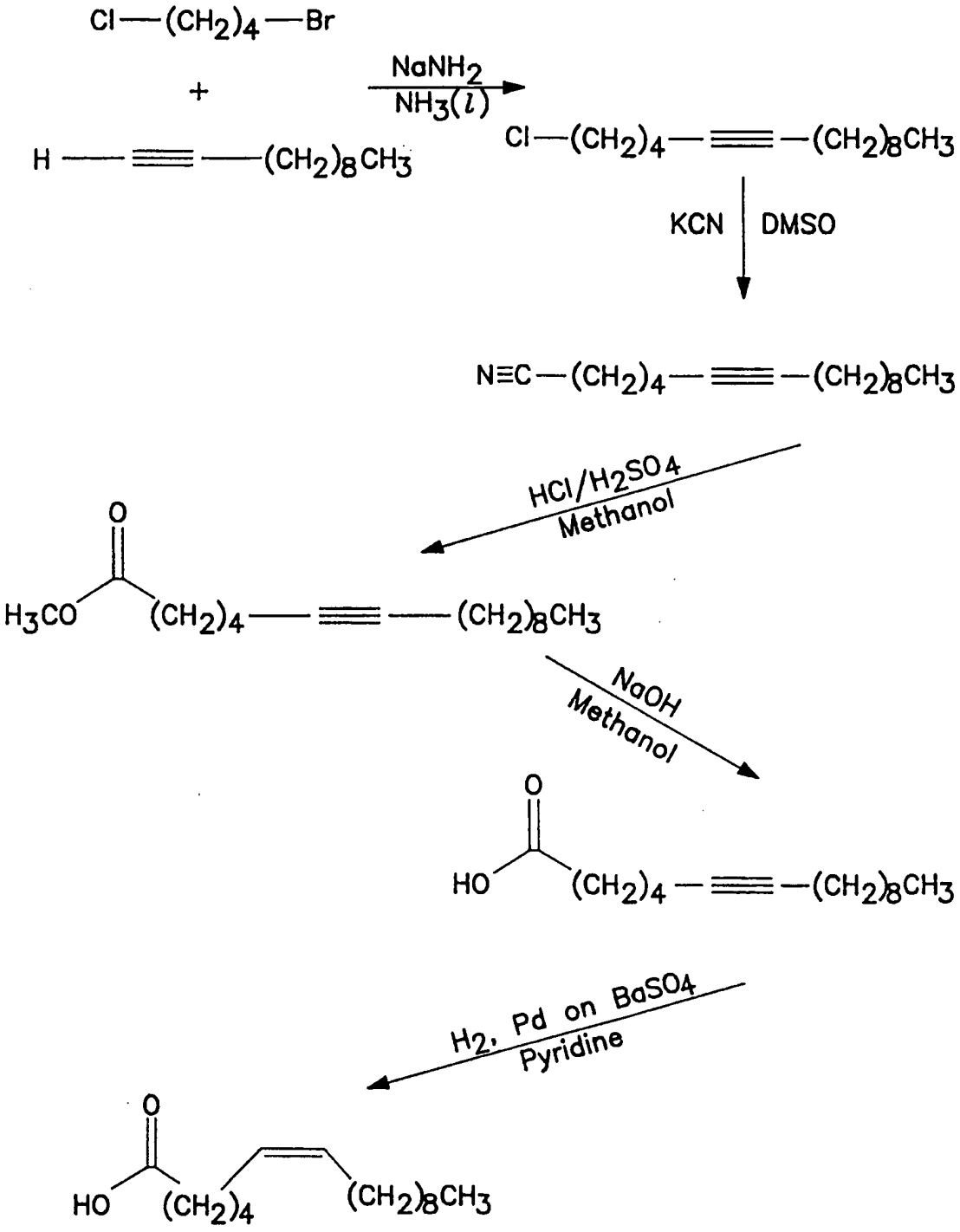

Cl—(CH2)4—Br
+
H—≡—(CH2)8CH3
NaNH2
NH3(l)
Cl—(CH2)4—≡—(CH2)8CH3
KCN
DMSO
N≡C—(CH2)4—≡—(CH2)8CH3
HCl/H2SO4
Methanol
H3CO
(CH2)4—≡—(CH2)8CH3
NaOH
Methanol
HO
(CH2)4—≡—(CH2)8CH3
H2, Pd on BaSO4
Pyridine
HO
(CH2)4
(CH2)8CH3

FIG. 1

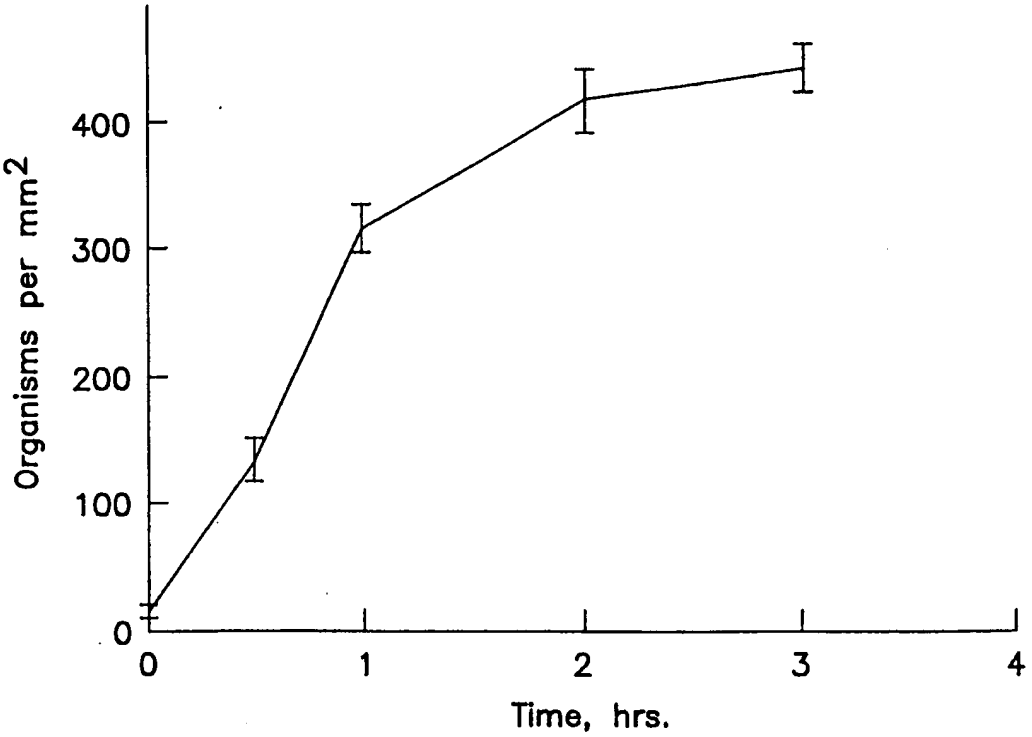

Organisms per mm2
400
300
200
100
0
0
1
2
3
4
Time, hrs.

FIG. 2

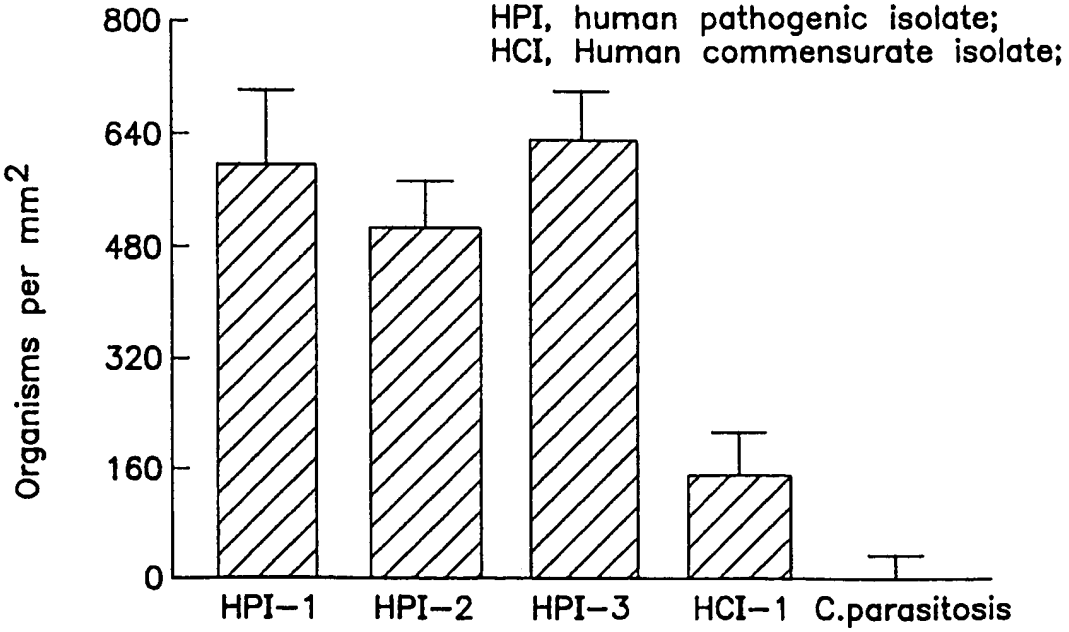

HPI, human pathogenic isolate;
HCI, Human commensurate isolate;
Organisms per mm2
800
640
480
320
160
0
HPI–1
HPI–2
HPI–3
HCI–1
C.parasitosis

FIG. 3

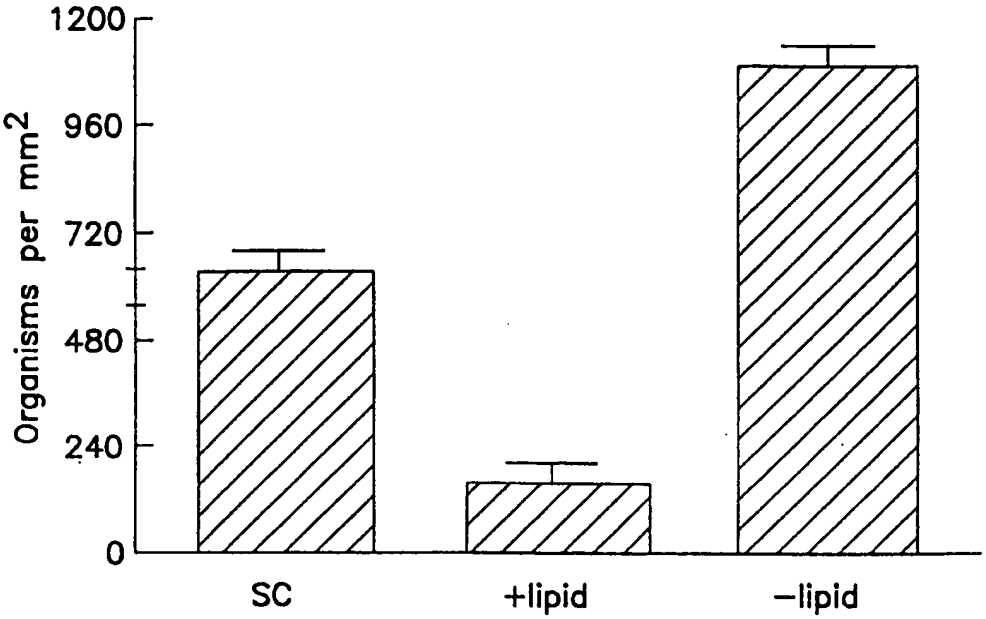

1200
960
720
480
240
0
Organisms per mm2
SC
+lipid
−lipid

FIG. 4

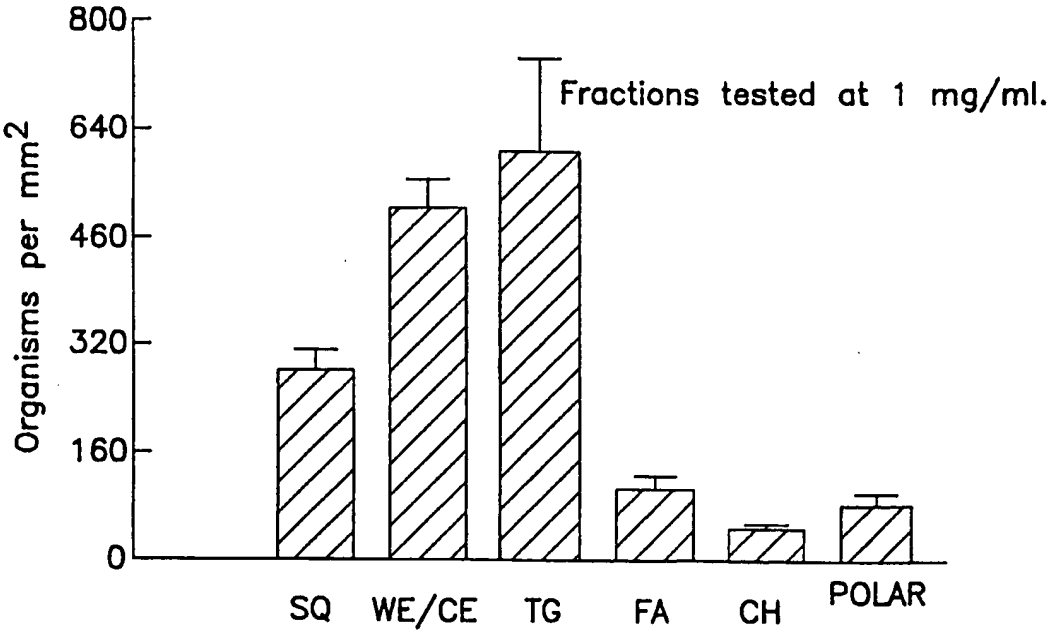

800
640
460
320
160
0
Organisms per mm2
Fractions tested at 1 mg/ml.
SQ
WE/CE
TG
FA
CH
POLAR

FIG. 5

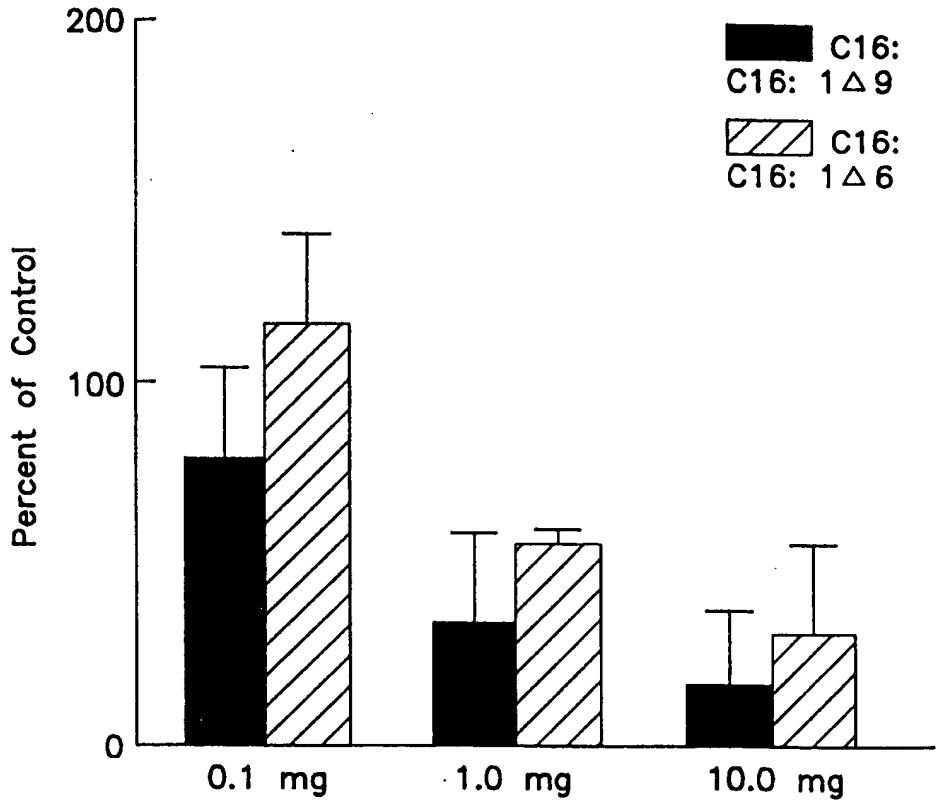

200
100
0
Percent of Control
C16:
C16: 1Δ9
C16:
C16: 1Δ6
0.1 mg
1.0 mg
10.0 mg

FIG. 6

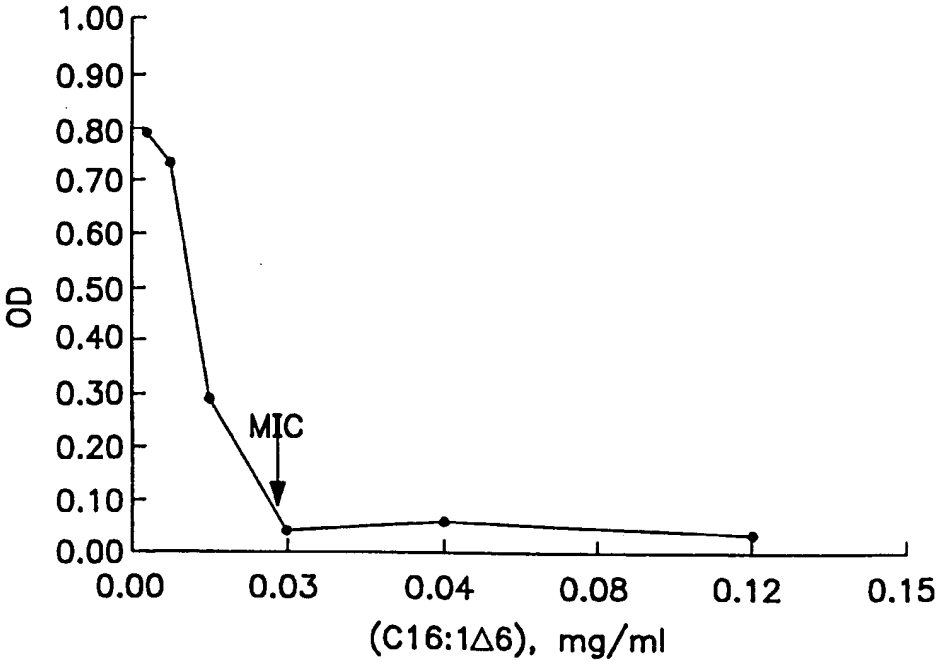

1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
OD
MIC
0.00
0.03
0.04
0.08
0.12
0.15
(C16:1Δ6), mg/ml

FIG. 7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/18826

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A01N 25/08
US CL :424/405

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. :

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

424/404, 405, 484; 514/560, 969

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 545,505 A (HURD) 03 September 1895, see entire document.	12-14, 17-19, 28, 29 --- 1-29
X --- Y	US 875,380 A (REUTER) 31 December 1907, see page 2 and claims.	1-5, 12-16, 28-29 --- 1-29
X --- Y	US 971,681 A (KNORF) 04 October 1910, see pages 1 and 2.	1-6, 12-27 --- 1-29

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 06 DECEMBER 1997	Date of mailing of the international search report 03 FEB 1998
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer NEIL LEVY Telephone No. (703) 308-2351

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/18826

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 3,883,661 A (YOUNG) 13 May 1975, see entire document.	1-29
Y	US 2,372,807 A (BROWN) 03 April 1945, see entire document.	1-29
Y	US 2,804,424 A (STIRN et al.) 27 August 1957, see entire document.	1-29

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Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Group I, claims 1-6, 8-24, 28 and 29 drawn to compositions, classified in class 424, subclass 405 with species of Toothpaste, mouthwash, shampoo hair care, skin ointment, deodorant, antiperspirant, and oral drug.

Group II, claims 7 and 25-27, drawn to transdermal devices, classified in class 604, subclass 290.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.